REMARKS

The Official Action dated September 7, 2001 and the references cited therein have been carefully reviewed. In view of the amendments presented herewith and the following remarks, Applicants respectfully request favorable reconsideration and allowance of the application.

Brief Summary of the Prosecution

The present invention is directed to novel compositions and methods for improving vanillin production in intact plants and culture cells or tissues of *Vanilla planifolia*. The invention provides transgenic plants and cells with improved vanillin production. For improving the production of vanillin in *V. planifolia*, a preferred method comprises providing a tissue culture of *V. planifolia* and supplementing the culture with malic acid, 3,4-dihydroxybenzaldehyde, citric acid, pyruvic acid, oxaloacetic acid, succinic acid, glycosylated lysozyme or any combination thereof, in an amount effective to improve the vanillin production as compared with cultures not supplemented with the compound. The invention also provides methods for increasing vanillin production more specifically by adding, for example, malic acid at between about 0.01-5%, or 3,4-dihydroxybenzaldehyde at 0.1-5 mM. The cultured *V. planifolia* cells produced by the methods are also provided.

In the Office Action dated September 7, 2001, the following rejections were made, or issues were raised.

1. Applicants' election of Group I claims was acknowledged, claims 12-30 were cancelled; and the Group I claims were renumbered 1-10 leaving those claims pending.

2. The Information Disclosure Statement filed January 10, 2000 was considered by the examiner.

3. A minor informality in claim 1 was identified.

4. Claims 1-10 were rejected under 35 U.S.C. §112, first paragraph as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to make and/or use the invention. In particular, the examiner cited Rao et al. (J Sci. Food Agric. 80:289-304, 2000) as alleged evidence of unpredictability in the art.

5. Claims 1, 7 and 9-10, and claims 2-6 and 8 dependent thereon were rejected under 35 U.S.C. §112, second paragraph as allegedly indefinite for failing to point out and distinctly claim the subject matter which applicants regard as the invention. Claims 1 uses the allegedly indefinite term "glycosylated lysozyme", claim 7 uses the allegedly indefinite term "glycoslyated lysozyme elicitor", and claim 9 uses the allegedly indefinite term "at least."

6. Claims 8-10 were rejected under 35 U.S.C. §102 (b) as allegedly anticipated by Knuth et al. (U.S. Patent No: 5,057,424).

Amendments made in this paper and submissions herewith:

In the present Amendment, the specification has been amended to correct minor errors and to achieve overall consistency throughout, and claims 1, 5, and 7 have been amended to correct typographical errors or to provide clarity. Claim 8 has been cancelled. New claims 31-40 have been added. Support can be found in the specification for any amendments to existing claims, and all new claims are fully supported by the specification. Applicants' undersigned representative states that the amendments to the specification and claims add no new matter, nor do the new claims.

Claim Objections:

Applicants acknowledge the informality noted by the examiner. This was due to a typographical error and has been corrected.

Rejections under 35 U.S.C. §112, first paragraph:

Claims 1-10 stand rejected under 35 U.S.C. §112, first paragraph as allegedly not enabling one skilled in the art to make and use the invention. The claims relate to methods for improving vanillin production in cultured *Vanilla planifolia* cells by supplementing the culture with malic acid, 3,4-dihydroxybenzaldehyde, citric acid, pyruvic acid, oxaloacetic acid, succinic acid, glycosylated lysozyme, or any combination thereof. The claims also relate to cultured *V. planifolia* cells produced by the methods of the invention. The examiner found that the specification is enabling for a method of improving vanillin production in *V. planifolia* by supplementing the culture with 3% malic acid alone, 1mM 3,4-dihydroxybenzaldehyde alone, or 30μg/ml glycosylated lysozyme elicitor protein alone. The examiner alleged that the specification does not teach whether any other compound will improve production of vanillin in cultured *V. planifolia*. The examiner cited Rao et al. (J Sci. Food Agric. 80:289-304, 2000) for the proposition "that different types of phytohormones and different types of vanillin precursors can positively or negatively affect the vanillin in cultured *Vanilla* cells." The examiner concludes from this one reference that there is unpredictability in the art and that it would require undue experimentation by one skilled in the art to make and use the claimed invention. Applicants respectfully traverse the rejection.

Before any analysis of enablement can occur, it is necessary for the examiner to construe the claims. The examiner should always look for enabled, allowable subject matter and communicate to applicants what that subject matter is at the earliest point possible in the prosecution of the application. (MPEP 2164.04)

The Federal Circuit has consistently held that "the specification must teach those of ordinary skill in the art how to make and use the full scope of **the invention** without undue experimentation. In re Wright, 999 F.2d 1557,1561(Fed. Cir. 1993). Since the invention is obviously that for which patent protection is sought, 'the claims must be analyzed first in order to determine exactly what subject matter they encompass. In re Angstadt, 537 F.2d 498,501 (CCPA 1976). The subject matter there set out must be presumed, in the absence of evidence to the contrary, to be that "which

the applicant regards as his invention"' Full effect must be given to all claim limitations. In re Angstadt, 537 F.2d 498,501.

In the present application, claim 1 and claims that depend from it are directed to a method of improving vanillin production wherein specific compounds are added to *Vanilla planifolia* cultures. These compounds include malic acid, 3,4-dihydroxybenzaldehyde, citric acid, pyruvic acid, oxaloacetic acid, succinic acid, glycosylated lysozyme and any combinations thereof, in an amount effective to improve vanillin production when compared with cultures which are not supplemented with the compound.

In general, the examiner should not use post-filing date references to demonstrate that the patent is non-enabling. Exceptions to this rule could occur if a later-dated reference provides evidence of what one skilled in the art would have known on or before the effective filing date of the patent application. In re Hogan, 559 F.2d 595, 605 (CCPA 1977). MPEP 2164.05(a).

The examiner cited Rao et al., a post-filing date review article which does not directly "provide evidence" of what one skilled in the art would have known, but instead merely puts forth assertions and interpretations of the evidence of what those skilled in the art knew at the time of the effective filing date.

Turning now to the substantive disclosures of Rao et al. relied upon by the examiner, in particular, applicants note that first of the sections the examiner cited relates to the influence of phytohormones on vanillin production. Therein, Rao et al. cite Knuth and Sahai that a hormone mix containing 2,4-D and benzyl adenine was necessary and useful in the initiation of callus and growth of *V. fragrans* and other species. No direct evidence of the effects of these compounds on *vanillin production* are cited by Rao et al. for this study. Rao et al further cite a study wherein napthaleneacetic acid with or without cytokinins were used resulting in significant increases in total extractable phenolics. Again, no evidence of the effect on vanillin production was provided. Rao et al additionally mention a study wherein kinetin was

successfully used as an elicitor to induce vanillic acid synthesis in cell suspension cultures of *V. planifolia*.

The claims clearly and precisely lay out the limitations of what applicants regard as the invention. None of these claims contain any element or require any limitation relating to 'phytohormones' or the use of the compounds cited by Rao et al.. The applicants respectfully submit that, with respect to these claims, the examiner has either misconstrued the claims or read into the claims a limitation which is not contained within them.

Applicants note that none of the compounds claimed in the present invention are considered to be 'phytohormones'. Additionally, Rao et al. do not cite any evidence to suggest unpredictability of any of the compounds claimed by the applicants. The present claims include no reference to phytohormones, or, in particular to the compounds 2,4-D, benzyl adenine, napthaleneacetic acid, cytokinins or kinetins. Furthermore, it appears from the evidence provided in Rao et al. that these compounds either increased vanillin production or that it cannot be determined what effect they had on vanillin production from the evidence. There is no evidence that any of the compounds claimed by applicants or cited by Rao et al. negatively affected the production of vanillin as examiner asserts. This section from the work of Rao et al. does support the examiners enablement rejection.

Aside from the phytohormones, the examiner apparently (it is unclear due to an apparent typographical error in examiner's page cite) also asserts that Rao et al. teach that "different types of vanillin precursors can positively or negatively affect the production of vanillin in cultured *Vanilla* cells."

Rao et al. cited a study wherein feeding cinnamic acid and ferulic acid to *V. planifolia* cultures resulted in formation of p-hydroxybenzoic acid and vanillic acid. They also cited a study in which conditioned medium, when used for culturing *V. planifolia* callus, resulted in a two-fold increase in vanillin production. Another study

cited showed feeding ferulic acid resulted in an increase by 1.7-fold compared to untreated cells.

Rao et al. also cited experiments on light wherein little or no differences were detected on growth or on concentrations of p-hydroxybenzoic acid, p-hydroxybenzyl alcohol, p-hydroxybenzaldehyde and p-coumaric acid. Rao et al. further cited Knuth and Sahai wherein adding phenylalanine and ferulic acid resulted in "little enhancement" whereas the addition of vanillyl alcohol resulted in significant increase of vanillin. Finally, Rao et al. cite the work of Westcott et al. studying the conversion of ferulic acid to vanillin. They used charcoal to adsorb vanillin from the culture as it was produced and this increased the production of vanillin by 5-10 times in comparison to cultures not provided with the ferulic acid precursor.

Again, the applicants' claims clearly and precisely lay out the limitations of what the applicants regard as their invention. None of these claims contain any element or require any limitation relating to cinnamic acid, ferulic acid, phenylalanine, or the use charcoal adsorbents or varying light intensity as cited by Rao et al.. The applicants respectfully submit that, with respect to these claims, the examiner has either misconstrued the claims or read into the claims a limitation which is not contained within them.

Applicants note that none of the compounds claimed in the present invention are those cited by Rao et al. Additionally, Rao et al. do not cite any evidence to suggest unpredictability of any of the compounds claimed by the applicants. Furthermore it appears from the "evidence" provided in Rao et al. that these compounds as well either increased vanillin production or it cannot be determined what effect they had on vanillin production from the evidence provided. There is no evidence that any of the compounds claimed by applicants or cited by Rao et al. can "negatively affect the production of vanillin" as examiner asserts. This section from the work of Rao et al. does support the examiners enablement rejection.

Significantly, the Patent Office Board of Appeals, when presented with similar facts, reversed an Examiner's rejection of claims that related to processes for using monoclonal antibodies to isolate and purify human fibroblasts. In that case, the examiner's rejection was based on alleged lack of enablement of a screening assay which was not required by the claims. The Board stated:

> The present disclosure as well as that of the parent application does enable one of ordinary skill in the art to practice the ***claimed*** invention. Thus, the claims on appeal are disclosed in the manner provided by 35 U.S.C. 112, first paragraph,. . . and we reverse this rejection of the claims.

Ex parte Erlich, 3 USPQ 2d 1011 (Pat. Off Bd. App. 1987) (emphasis in original).

Moreover, the examiner has the initial burden to establish a reasonable basis to question the enablement provided for the claimed invention. (MPEP 2164.04) In this instance, the examiner has stated no reason to doubt the objective truth of the statements contained within the specification and which enable the claimed invention. The examiner provides only the post-filing reference to Rao et al. which allegedly relates to demonstrating unpredictability in the art. Further, the examiner claims that undue experimentation would be required to make and use the applicants' claimed invention.

With respect to the present application, the specification teaches methods of improving vanillin production in *V. planifolia* tissues and cultures. The specification teaches addition of specific compounds to cultures of *V. planifolia*. The specification also teaches presently preferred concentrations or amounts of the enumerated compounds to be added. In addition, the specification sets forth multiple examples of improving vanillin production through additions of these compounds alone and in combination with one or more other compounds, or in combination with specific stressors are also taught in the specification. The use of these compounds, conditions and combinations to elicit improved vanillin production is both novel and surprising.

The specification teaches the methods for cultures varying in age from 0 –1 month, including clusters, embryo cultures and tissue homogenates.

For example, the specification (p 24) Example 3 teaches that "Proaldehyde at different concentrations was added to the medium, either alone **or in combination** with the following treatments: (emphasis added)

–malic acid (0.01-3.0%)

–varying pH of the medium

–varying ascorbic acid concentration

–varying temperatures, including cold and heat.

In Example 4, the specification (p 25) teaches:

"Malic acid was applied to the following: (1) intact roots, (2) intact shoots, (3) embryo cultures, (4) cluster cultures, and (5) cuttings. The age of the cultures were between 0 and 1 month. Malic acid was applied alone or in combination with the following: starvation without sugar (sucrose); shear stress induced by bioreactor impeller; citric acid; varying concentrations of oxygen andethylene; oxaloacetic acid (sodium salt); ascorbic acid; pyruvic acid; glutamic acid; succinic acid; or salt stress.

Adding proaldehyde for a few days, followed by addition of malic acid, was found to increase production of vanillin and vanillyl alcohol. If sucrose is omitted from the malic acid treatment (i.e. starvation due to lack of sucrose), the onset of vanillyl alcohol production occurs more quickly."

Furthermore, the specification details routine methods for the culture of the *V. planifolia* and of the embryo tissue, as well as routine methods of analysis of the various metabolic intermediates. Finally the specification puts forth the proposed pathway and constraints on the biosynthesis of vanillin. Collectively these teachings readily allow one of skill in the art to make and use the claimed invention, in

particular the teachings would allow one of skill in the art to add compounds alone or in combination with each other to a culture of *V. planifolia* to improve vanillin production.

Applicants assert, based on the above detailed response, that undue experimentation is not required. While some experimentation may be useful in specific circumstances, the quantity of experimentation is not dispositive of the analysis (MPEP 2164.04). The key word is "undue," not "experimentation". In re Angstadt, 537 F.2d 498,504 (CCPA 1976). Further, the scope of enablement must only bear a reasonable connection to the scope of the claims. See, e.g., In re Fisher, 427 F.2d 833,839 (CCPA 1970). Applicants further assert that Examiner's rejection is improperly based on "phytohormones" and the use of specific vanillin precursors (e.g. cinnamic acid, ferulic acid, phenylalanine) when such a limitation is neither part of the claimed invention nor necessary to make and use the invention claimed. Ample working examples are provided, despite that the specification need not contain a working example if the invention is otherwise disclosed in such a manner that one skilled in the art will be able to practice it without an undue amount of experimentation. In re Borokowski, 422 F.2d 904,908 (CCPA 1970). In the specification at hand, multiple working examples are provided which each teach how to make and use the invention, including examples which teach how to prepare and grow the V. planifolia cultures, specifically how to assay the desired metabolic products and intermediates, and working ranges of concentration or amount of compounds to add, and what to avoid. Additionally applicants provide information as to preferred additions and combinations. With this information in hand, one of skill in the art can make and use the invention either to improve vanillin production in cultures of *V. planifolia* or to produce such cultures.

While a certain amount of experimentation may be useful, particularly for optimizing or maximizing, it is not undue as discussed above. The combined teachings of the specification based on the whole of the evidence show that the specification is indeed enabling for the claimed invention. The specification provides

more than adequate guidance to practice the claimed invention. The specification need not be conclusive, but merely convincing to one skilled in the art. (MPEP 2164.05)

Applicants assert that the typical skill of those in the art is high, but applicants respectfully disagree that the art with respect to the claimed invention is unpredictable. It would require only routine experimentation to add the claimed compounds to a culture and then to measure the vanillin production by the routine method provided or known to those skilled in the art. There is nothing that requires any undue experimentation or going beyond what is taught in the specification.

Where the claimed invention is the application of an unpredictable technology in the early stages of development, an enabling description must provide those skilled in the art with a specific and useful teaching. Genentech v. Novo Nordisk, 108 F.3d 1361,1367-1368 (Fed. Cir. 1997). And while tossing out the mere germ of an idea does not constitute enabling disclosure, every aspect of a generic claim certainly need not have been carried out by an inventor, or exemplified in the specification so long as reasonable detail is provided to enable the invention to be understood and carried out. Enzo Biochem v. Calgene, 188 F.3d 1362,1374 (Fed. Cir. 1999).

In the present application, the art of adding compounds to a culture for improvement of a metabolic pathway is not an unpredictable technology "in the early stages of development". Rather, the addition of such compounds and even the optimization of the parameters are well within the grasp of the skilled artisan in this area.

For the foregoing reasons, applicants respectfully request the withdrawal of these rejections under 35 U.S.C. Sec. 112, first paragraph. Applicants submit that the examiner has not provided any evidence that supports the allegation as to unpredictability in the art. The examiner has cited only one reference which cannot support the enablement rejection with respect to the claimed invention. There is no limitation in the claims with respect to phytohormones or the compounds cited by Rao

et al.. Applicants assert that the specification is fully enabling for the claimed invention. Any experimentation which is required is not undue. Accordingly, applicants respectfully request reconsideration and withdrawal of the Examiner's rejection as to these claims which are fully enabled. In addition, and for all of the reasons set forth above, the rejections also do not apply to new claims 31-40, which are believed to be in condition for allowance.

Rejections under 35 U.S.C. §112, second paragraph:

Claims 1 and 7 are rejected as allegedly indefinite for use of the terms "glycosylated lysozyme" and "glycosylated lysozyme elicitor", respiectively. The language has been clarified and made consistent throughout the specification and the claims, so as to obviate the grounds of this rejection. The applicants therefore respectfully request that the rejection be withdrawn.

With respect to the examiner's rejection of claims 9-10 for use of the phrase "at least", applicants traverse this rejection and assert that the meaning of "at least" is clear as used in claim 9 ". . .*at least* twice as much vanillin as equivalent cultured cells no supplemented with the compounds." It would be completely clear to one skilled in the art based on the common meaning of the term and particularly in light of the specification. The phrase clearly establishes a lower limit; it simply means that the claimed culture would have two times as much, *or more*, vanillin as an untreated culture under the same conditions. Likewise in the case of claim 10 ". . .*at least* ten times as much vanillin as equivalent cultured cells no supplemented with the compounds" simply means ten times as much, *or more*, vanillin as an untreated culture under the same conditions. The phrase "at least" is not relative terminology (MPEP 2173.05(b)) nor is it being used in any manner repugnant to its usual meaning (MPEP 2173.05(a)). As well, for new claims 31-40 applicants assert that the meaning of "at least", where used therein, is clear to one of skill in the art.

Rejections under 35 U.S.C. §102:

Claims 8-10 stand rejected as allegedly anticipated by Knuth et al. (U.S. Patent Number 5,057,424). Claim 8 is cancelled, thereby rendering its rejection moot. A claim is anticipated by a reference only if each and every element of the claim is found, either expressly or inherently, in that reference. (MPEP 2131). Moreover, the identical invention must be shown in as complete detail as is contained in the claim. Id. Under these standards, applicants assert that Knuth et al. do not anticipate the claims as amended or the newly-added claims.

While Knuth et al. disclose *Vanilla* cells which are grown in culture, which do not form identifiable differentiated cell structures, and which can secrete vanillin into the medium, they do not disclose any embryo cultures *per se*, nor do they teach cell cultures with the addition of the supplements or elicitors as defined by the claims in the instant invention. Moreover, Knuth et al. do not teach cultures with specific limitations on the amount of increased vanillin production required to be present. Therefore, Knuth et al. cannot serve as the basis for an anticipation rejection for the claims as amended.

Specifically, the disclosure of Knuth et al. does not address or consider the further limitation of claim 9 and claim 10. Claim 9 contains the further limitation "wherein the cells produce at least twice as much vanillin as cells cultured under equivalent conditions but which were not supplemented with the compounds." The limitation in claim 10 is to "ten times as much vanillin". Since Knuth et al. do not teach cultures which are limited to specific levels of increased production as in the present claims, the reference cannot serve to anticipate the claims. Furthermore, Knuth et al., while disclosing in column 15, lines 55-60, a culture which produces vanillin over time, do not anticipate the subject matter of the present claims because varying the time a culture is incubated would be readily recognized by those skilled in the art as modifying the culture conditions. The claims of the instant invention expressly limit culture to producing the required increase in vanillin (twice as much or ten times as much) in comparison to a culture grown under equivalent conditions but

without the additional compounds or supplements. There is no teaching or suggestion in Knuth et al. to accomplish this. Furthermore, the teachings of Knuth et al., as they may broadly relate to the present invention, are certainly not presented in the same level of detail. The present invention as claimed, including the new claims is not anticipated by this limited disclosure of Knuth et al. wherein the amount of vanillin in a culture increases over time. Again the examiner is either misconstruing the limitations of the present invention, or reading into the claims a limitation which is not present. Claim 8 has been cancelled to satisfy the examiners rejection of the broadest claim relating to the cultured *Vanilla* cells. The claims as amended are not anticipated by the limited disclosure of Knuth et al. and the applicants respectfully request that the rejection be withdrawn to amended claims 9 and 10, and further that the rejection not be applied to new claims 31-40.

In light of the foregoing arguments and for all the reasons laid out above, the applicants respectfully assert that all claims as amended are novel over the cited reference. Accordingly, withdrawal of the rejections under 35 U.S.C. Sec. 102(b) Knuth et al. is requested.

Summary

In view of the foregoing amendments and remarks, the applicants submit that this application is in condition for allowance and respectfully request early and favorable notification to that effect. If it would expedite prosecution of this application, the Examiner is invited to confer with applicants' undersigned representative.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned **"Version with markings to show changes made."**

Respectfully submitted,

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Date: March 7, 2002

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

The paragraph beginning at page 3, line 35, has been amended as follows:

In preferred embodiments of the invention, the tissue culture is an embryo culture. In another preferred embodiment, the culture is supplemented with malic acid at a concentration of between about 0.01% and 5% by weight of the culture medium. In another preferred embodiment, the culture is supplemented with 3,4-~~dihyrdoxybenzaldehyde~~ dihydroxybenzaldehyde at a concentration of between about 0.1 and 5 mM. In another embodiment, the culture is supplemented with about 0.01 to about 5% by weight of a compound selected from the group consisting of succinic acid, oxaloacetic acid, citric acid and pyruvic acid. In yet another embodiment, the culture is supplemented with about 1 to about 100 µg/ml of ~~a~~ glycosylated lysozyme ~~elicitor~~.

The paragraph beginning at page **5**, line **29**, has been amended as follows:

According to yet another aspect of the invention, a method for improving vanillin accumulation in cell or tissue culture of *Vanilla planifolia* is provided, which comprises inhibiting production or activity of vanillyl alcohol dehydrogenase in cells comprising the cell or tissue culture, the inhibition resulting in the improved vanillin accumulation. In one embodiment, the inhibiting comprises genetically engineering the cells to inhibit expression of a gene encoding the vanillyl alcohol dehydrogenase. In another embodiment, the inhibiting comprises treating the culture with an inhibitor of vanillyl alcohol dehydrogenase activity. Cultures produced by the aforementioned method are also provided.

The paragraph beginning at page **11**, line **4**, has been amended as follows:

The enzyme catalyzing the conversion of vanillin to vanillyl alcohol has been determined to be an alcohol dehydrogenase, which the inventors have named vanillyl alcohol ~~dehyrdogenase~~ dehydrogenase (VAD). The purification of VAD from cultured cells of *V. planifolia* and its characterization are described in Example 8.

The paragraph beginning at page **12**, line **12**, has been amended as follows:

Another useful elicitor of vanillin production in cultured vanilla is ~~the~~ glycosylated ~~lysozayme~~ lysozyme, a protein elicitor described in U.S. Patent No. 5,552,307 to Kessler et al. As shown in Example 5, treatment with this elicitor more than doubles the amount of vanillin produced in cultured vanilla cells.

The paragraph beginning at page **12**, line **18**, has been amended as follows:

Another elicitor of vanillin production in cultured cells is heat stress, i.e. placing the cultures at 33-37 °C for an extended period of time. Heat stress of this nature has been found to increase production of vanillin and related compounds in cultured cells by at least ~~2-3-fold~~ 2-3-fold. ~~Somilarly~~ Similarly, shear stress, as described in greater detail in the examples, increases production of vanillin and related compounds in cultured cells by at least ~~2-3-fold~~ 2-3-fold.

The paragraph beginning at page **14**, line **7**, has been amended as follows:

The next key enzyme in the vanillin biosynthetic pathway is the oxygenase that catalyzes hydroxylation of p-hydroxybenzyl alcohol to 3,4-dihydroxybenzyl alcohol. This enzyme is believed to be a cytochrome P450 monooxygenase, and this step is believed to be the rate-limiting step in the vanillin biosynthetic pathway in

cultured cells. For these reasons, up-regulation or some other form of ~~supplentation~~ supplementation of this enzyme in cultured cells and in intact plants.

The paragraph beginning at page **25**, line **16**, has been amended as follows:

Malic acid was applied to the following: (1) intact roots, (2) intact shoots, (3) embryo cultures, (4) cluster cultures, and (5) cuttings. The age of the cultures were between 0 and 1 month. Malic acid was applied alone or in combination with the following: starvation without sugar (sucrose); shear stress induced by bioreactor impeller; citric acid; varying concentrations of oxygen ~~andethylene~~ and ethylene; oxaloacetic acid (sodium salt); ascorbic acid; pyruvic acid; glutamic acid; succinic acid; or salt stress.

The paragraph beginning at page **28**, line **45**, has been amended as follows:

The table below shows the results of experiments testing the effect of ~~the~~ glycosylated lysozyme, an elicitor ~~proteins~~ protein described in U.S. Patent No. 5,552,307 on vanillin precursors in vanilla embryo cultures. As can be seen, these proteins were effective in stimulating vanillin production in the cultured cells.

The paragraph beginning at page **28**, line **45**, has been amended as follows:

crude enzyme extract:	~~10µ~~ 10µl
substrate	100µl ~~(1.8 mM p-~~

~~coumarate in 0.1 M~~

~~Tris/HCl, pH 8.0~~

~~containing 10 mM DTT)~~

(substrate=1.8 mM p-coumarate in 0.1 M Tris/HCl, pH 8.0 with 10 mM DTT)

IN THE CLAIMS:

The claims have been amended as follows:

1. (Amended) A method for improving production of vanillin in cultured ~~*Vanillin*~~ *Vanilla planifolia*, which comprises:

a) providing a tissue culture of said *Vanilla planifolia*; and

b) supplementing the culture with a compound selected from the group consisting of malic acid, 3,4-dihydroxybenzaldehyde, citric acid, pyruvic acid, oxaloacetic acid, succinic acid, glycosylated lysozyme, and any combination thereof, in an amount effective to improve the vanillin production as compared with cultures not supplemented with the compound.

5. (Amended) The method of claim 1, wherein the culture is supplemented with ~~3,4-dihyrdoxybenzaldehyde~~ 3,4-dihydroxybenzaldehyde at a concentration of between about 0.1 and 5 mM.

7. (Amended) The method of claim 1, wherein the culture is supplemented with about 1 to about 100 µg/ml of glycosylated lysozyme ~~elicitor~~.

9. (Amended) ~~The cultured~~ Cultured *Vanilla planifolia* cells ~~of claim 9~~, produced by the method of claim 1, ~~which~~ wherein the cultured cells produce

at least twice as much vanillin as ~~equivalent cultured cells~~ cells cultured under equivalent conditions but which were not supplemented with the compounds.

10. (Amended) ~~The cultured~~ Cultured *Vanilla planifolia* cells of claim 9, ~~which~~ wherein the cultured cells produce at least ten times as much vanillin as ~~equivalent cultured cells~~ cells cultured under equivalent conditions but which were not supplemented with the compounds.